Title

Modification M007 of analytical method 01387 for the determination of fluoxapiprolin (BCS-CS55621) in drinking and surface water by HPLC-MS/MS

- Final Report -

Test Item

Fluoxapiprolin (BCS-CS55621)

Data Requirements/Guidelines

- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
- Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010
- European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00
- US EPA OCSPP 850.6100

Completion Date

2019-08-23
(yyyy-mm-dd)

2 Introduction and Objective

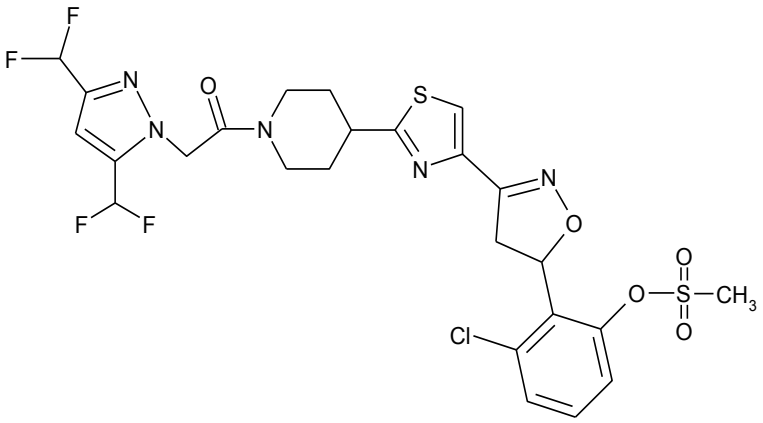
The objective of the study was to develop and validate the analytical method 01387/M007 for the determination of concentrations of fluoxapiprolin (BCS-CS55621) in drinking and surface water by HPLC-MS/MS.

3 Compound

3.1 Reference Item

Generally, only sufficiently characterised and certified substances were used as reference item.

Fluoxapiprolin:

Common name:	Fluoxapiprolin
Chemical code:	BCS-CS55621
Chemical name:	2-{3-[2-(1-{[3,5-bis(diflouromethyl)-1H-pyrazol-1-yl]acetyl}piperidin-4-yl)-1,3-thiazol-4-yl]-4,5-dihydro-1,2-oxazol-5-yl}-3-chlorophenyl methanesulfonate
CAS no.:	1360819-11-9
Mol ID:	25996
Structural formula:	
Empirical formula:	$C_{25}H_{24}ClF_4N_5O_5S_2$
Molecular weight:	650.07 g/mol
Solubility in water:	0.08 mg/L (20 °C) at pH 5.9
Appearance:	light beige powder
Certificate No.:	AZ 21323, dated on 2017-01-16
Purity:	98.2 %
Origin batch No.:	NLL 9039-13-1
Expiry date:	2020-01-12

4 Experimental Section

4.1 Test System

The analytical method was validated for surface water. A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L.

For method validation surface water from the river Wupper sampled in Leverkusen-Opladen was used. Characteristics of the test system are listed in Table 3.

Table 3: Characteristics of the Surface Water From River Wupper, Sampled on 2015-01-13 in Leverkusen Opladen (Germany)

Parameter Wupper water	Value
Total organic carbon (TOC)	<2 mg/L
Dissolved organic carbon (DOC)	-
Conductivity	273 µS/cm
pH	7.5
Water hardness	4.6°dH
Filterable solids	<5 mg/L
Dry residue after filtration	170 mg/L

4.2 Safety

The German guidelines for laboratories of the Employees' Liability Insurance Association, e.g. Working Safely in Laboratories [4] or comparable guidelines in other countries should be observed.

The following chemicals were used, which are classified by the hazardous material regulations. The classification is based on the Globally Harmonised System (GHS) [5].

Substance	Signal word	Hazard statements
Methanol	Danger	H225 Highly flammable liquid and vapour H301 Toxic if swallowed H311 Toxic in contact with skin H331 Toxic if inhaled H370 Causes damage to organs
Acetonitrile	Danger	H225 Highly flammable liquid and vapour H302 Harmful if swallowed H312 Harmful in contact with skin H319 Causes serious eye irritation H332 Harmful if inhaled
Formic acid	Danger	H226 Flammable liquid and vapour H314 Causes severe skin burns and eye damage
Ammonium formate	Warning	H315 Causes skin irritation H319 Causes serious eye irritation H335 May cause respiratory irritation
Fluoxapiprolin (BCS-CS55621)		Caution – not fully tested yet

The pertinent safety instructions must be observed when working with all compounds mentioned in this method.

4.3 Materials

4.3.1 Apparatus and Reagents

For apparatus and reagents please see Appendix 2.

4.3.2 Stock Solutions

The stock solution was prepared by weighing a defined amount of the reference item into a volumetric flask and making up to volume with acetonitrile/water (8/2, v/v).

Table 4: Preparation Scheme of Reference Item Stock Solutions

Reference Item No.		Mass [mg]	Volume [mL]	Solvent	Final Concentration Actual*1 [mg/L]
A1	Fluoxapiprolin (BCS-CS55621)	5.18	10	*2	508.7

*1: Concentration is corrected for purity

*2: Acetonitrile/water, 8/2, v/v

4.3.3 Standard Solutions/Calibration Standards

Standard solutions (secondary standards) were prepared from the stock solution by dilution with river Wupper water/acetonitrile (8/2, v/v) and deionized water/acetonitrile, 8/2, v/v and acetonitrile/water (8/2, v/v).

Table 5: Preparation Scheme for Standard Solutions (Secondary Standards)

No.	Target Concentration (µg/L)	Prepared by Removal of [mL]	No. of Solution	Dilution to [mL]	Solvent
A2	Fluoxapiprolin (BCS-CS55621) (5010)	0.197	A1	20	*3
A3	Fluoxapiprolin (BCS-CS55621) (5.01)	0.020	A2	20	*1
A4	Fluoxapiprolin (BCS-CS55621) (1.00)	0.020	A2	100	*1
A5	Fluoxapiprolin (BCS-CS55621) (0.501)	2.00	A3	20	*1
A6	Fluoxapiprolin (BCS-CS55621) (0.100)	0.200	A3	10	*1
A7	Fluoxapiprolin (BCS-CS55621) (0.0501)	0.200	A3	20	*1
A8	Fluoxapiprolin (BCS-CS55621) (0.015)	0.150	A3	50	*1
A9	Fluoxapiprolin (BCS-CS55621) (501)	1.00	A2	10	*3
A10	Fluoxapiprolin (BCS-CS55621) (0.501)	0.020	A2	200	*1
A11	Fluoxapiprolin (BCS-CS55621) (0.501)	0.020	A2	200	*2

*1: River Wupper water/acetonitrile, 8/2, v/v
*2: Deionized water/acetonitrile, 8/2, v/v
*3: Acetonitrile/water, 8/2, v/v

4.4 Sample Preparation

The water samples are added with acetonitrile (25% of the water sample volume) and directly injected into the HPLC or after appropriate dilution with surface water/acetonitrile (80/20, v/v).

4.5 Instrumental Analysis

4.5.1 Principle of Measurement

An aliquot of the sample solution was injected into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). The pseudo molecular ions of the analytes ($[M+H]^+$, $[M-H]^-$ or any adducts) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation.

4.5.2 Variations in Instrument Conditions

Variations in equipment or sample characteristics and/or deterioration of system performance may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Instrument parameters and mobile phase may be adjusted to improve separation from unexpected interfering peaks.
Therefore, the given HPLC-MS/MS parameters listed (cf. Appendix 3) may require adaptation.

4.5.3 Chromatography

Instrument:	Agilent 1290 Binary Pump G 4220A Agilent 1200 Binary Pump G1312B (used as isocratic pump) Agilent Technologies (or equivalent)
Oven:	Aμ Mass HPLC Mini Column Oven Type μOV C100-17FR Aμ Mass Thermo Controller DTC-02 (or equivalent)
Column Pre-Heater:	Aμ Therm HPLC Eluent Preheater Type μOV P160-6/1.6 Aμ Mass Thermo Controller DTC-02 (or equivalent)
Autosampler:	PAL System RTC PAL CTC Analytics AG (or equivalent)
Column:	Ascentis Express 2.7 µm C18, 50 mm x 2.1 mm, Supelco (or equivalent)
Flow:	0.5 mL/min (for binary and iso pump)
Injection volume:	50 µL
Oven temperature:	80 °C
Pre heater temp.:	80 °C

Mobile phase: Bin Pump A: Deionized water / formic acid (1000/0.120, v/v) + 10 mM ammonium formate
Bin Pump B: Methanol / formic acid (1000/0.120, v/v) + 10 mM ammonium formate
Iso Pump C:
Deionized water / methanol / formic acid (500/500/0.120, v/v/v)

Retention time: Fluoxapiprolin (BCS-CS55621): approx. 2.6 min

Time Table:

Time [min]	A [%]	B [%]	Into MS	Into Waste
0.0	95	5	Iso pump	Bin pump
0.1	95	5		
1.0			Bin pump	Iso pump
3.0	5	95		
4.0	5	95	Iso pump	Bin pump
4.1	95	5		
5.0	95	5		

4.5.4 Detection

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo Ion Spray (ESI) interface operated in the positive ion mode and multiple reaction monitoring (MRM). Unit mass resolution was established and maintained in the mass resolving quadrupoles by maintaining a full width at half-maximum (FWHM) of about 0.7 amu. Optimal collisionally-activated dissociation (CAD) conditions for fragmentation of the pseudo molecular ions of the analytes were applied with nitrogen as the collision gas.

Detector: Triple Quadrupole Tandem Mass Spectrometer, AB Sciex API 5500, Windows 7, Analyst 1.6.2 software versions or any equivalent HPLC-MS/MS System

Interface: Turbo Ion Spray (ESI)

Scan Type: MRM (Multiple Reaction Monitoring)

Table 6: MS/MS Parameters for the Determination of Fluoxapiprolin (BCS-CS55621)

	Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)	Dwell Time (msec)	Collision Energy (eV)	Polarity
(BCS-CS55621) quantitation	650	610	100	29	pos
(BCS-CS55621) confirmation	650	193	100	47	pos

Note: Different MS/MS-instruments may result in different MRM transitions or signal intensity.

4.6 Calculation

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may substitute the equations used below. Calculations were done by using rounded values. Thus, rounding “errors” may occur if recalculations are made using the listed data.

4.6.1 Calculation of Concentrations

The measured concentration is calculated by comparison of the analyte response to a standard calibration curve (1/x weighted).

The following equation is used for calculation in case of a linear calibration curve of type y = ax + b:

$$C_S = \frac{A_{(S)}\text{Sample} - \text{Intercept (b)}}{\text{Slope (a)}} \times \text{Dilution factor}$$

C_S = concentration of the analyte in the sample
$A_{(S)}$Sample = peak area of the analyte in the sample solution
Intercept = point where the calibration curve crosses the y-axis
Slope = slope of the calibration curve

The calculation of concentrations is also possible by comparison of the peak areas of the samples with the peak areas of the external standard solutions.

The concentration in water samples can be calculated according to the following equation:

$$\text{Conc}_{\text{Sample}}\,[\mu g/L] = \frac{\text{Peak Area}_{\text{Sample}} \times \text{Conc}_{\text{Standard}}[\mu g/L] \times \text{Dilution Factor}}{\text{Peak Area}_{\text{Standard}}}$$

$\text{Conc}_{\text{Standard}}$ = concentration of the analyte in the standard solution
$\text{Conc}_{\text{Sample}}$ = concentration of the analyte in the sample solution

4.6.2 Calculation of the Repeatability (Precision)

The repeatability or precision of the method is defined as the dispersion of the validation results and is expressed as the relative standard deviation (RSD).

At each fortification level, the relative standard deviation was calculated as follows:

$$\text{Relative Standard Deviation}\,[\%] = \frac{\text{Standard Deviation}}{\text{Mean of Peak Area}} \times 100$$

5 Results and Discussion

5.1 Selectivity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two MRM transitions were monitored for the analytes. No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens.

5.2 Linearity

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water ranging from 0.015 µg/L to 5.0 µg/L (corresponding to 0.0188 µg/L to 6.25 µg/L in test water) for fluoxapiprolin (BCS-CS55621). The correlation coefficient was ≥ 0.9999 for both MRM transitions (see Appendix 5).

5.3 Untreated Control Samples

Two untreated control samples were examined. The concentrations were below 30% of the LOQ of 0.0625 µg/L for fluoxapiprolin (BCS-CS55621) (see Appendix 6).

Table 7: Apparent Concentrations in Control Samples (LOQ = 0.0625 µg/L)

Control Material		**Fluoxapiprolin (BCS-CS55621) Concentration [µg/L]**
Surface water	Sampled in Leverkusen-Opladen on 2015-01-13	< 0.3 x LOQ

5.5 Matrix Effects

The MS/MS detection of fluoxapiprolin (BCS-CS55621) was not affected by the matrix. The peak areas of the quantification and confirmatory ion in a surface water sample containing 0.5 µg/L (10 x LOQ) show no difference to the corresponding peak areas in deionized water.

5.6 Storage Stability

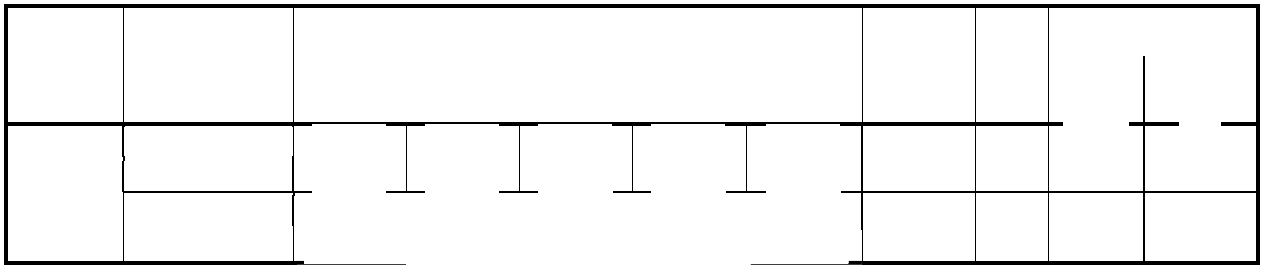
For examination of the storage stability of fluoxapiprolin (BCS-CS55621), different samples of surface water were fortified with 0.5 µg/L of fluoxapiprolin (BCS-CS55621). A set of three samples and two control samples were analysed as initial analysis at day 2 in order to confirm the nominal concentrations. The other samples were stored in a freezer at ≤ -18 °C. A set of three samples and two control samples was analysed after 8 days of storage. Before analysis the samples (20 mL) were added with 5 mL acetonitrile.

The results show that under freezer conditions at ≤ -18 °C or below fluoxapiprolin (BCS-CS55621) were stable for a storage period of 8 days. (see Table 9).

Table 9: Storage Stability of Fluoxapiprolin (BCS-CS55621) for the Quantitation Ion (m/z 650 → m/z 610) in Surface Water

Sample Material	FL [µg/L]	Storage Period	Concentration [µg/L] (Single Values)			Mean [µg/L] per FL	RSD [%]
Surface water	0.50	initial analysis	0.544	0.565	0.542	0.550	2.05
		8 days reanalysis	0.595	0.603	0.524	0.574	6.81

FL: Fortification Level, RSD: Relative Standard Deviation



Appendix 1:
Method characteristics

Table 11: Summary Parameters for the Analytical Method Used for the Quantitation of Fluoxapiprolin (BCS-CS55621) Residues in Surface Water (DER TABLE B.1.1)

Method ID	**01387/M007**
Analytes	fluoxapiprolin (BCS-CS55621)
Extraction solvent/technique	None – direct sample injection
Clean-up strategies	None – direct sample injection
Instrument/Detector/Column	Agilent 1290 Binary Pump and 1200 Binary Pump SL AB Sciex API 5500 with mass selective detector (MS/MS) Ascentis Express C18 2.7 µm, 50mm x 2.1 mm id
Standardization method	Linear regression with 1/x weighting
Storage stability	at least 8 days at ≤ -18 °C
Retention time	fluoxapiprolin (BCS-CS55621): approx. 2.6 min

Table 12: Characteristics for the Analytical Method Used for the Quantitation of Fluoxapiprolin (BCS-CS55621) Residues in Surface Water (DER TABLE C.1.2)

Analyte	fluoxapiprolin (BCS-CS55621)
Equipment ID	Agilent 1200LC, 1290LC, AB Sciex API 5500 HPLC-MS/MS
Limit of quantitation (LOQ)	Surface water: fluoxapiprolin (BCS-CS55621): 0.05 µg/L
Precision	The relative standard deviation for the peak area counts is ≤ 1.6% for both MRM transitions.
Reliability of the Method/[ILV]	An ILV has not been performed on this method.
Linearity	The method/detector response was linear (correlation coefficient r = ≥ 0.9999 for both MRM transitions) within the range of 0.015 µg/L to 5.0 µg/L for fluoxapiprolin (BCS-CS55621)
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

Appendix 2:
Apparatus and Reagents

Apparatus

- Liquid chromatograph, 1290 binary pump, 1200 binary pump, Agilent Technologies, Böblingen, Germany or equivalent
- Aμ Mass HPLC Mini Column Oven, Type μOV C100-17FR, Aμ Mass Thermo Controller DTC-02, 51379 Leverkusen, Germany or equivalent
- Aμ Therm HPLC Eluent Preheater, Type μOV P160-6/1.6, Aμ Mass Thermo Controller DTC-02, 51379 Leverkusen, Germany (or equivalent t
- Autosampler, RTC PAL, CTC Analytics, Zwingen, Switzerland or equivalent
- Mass spectrometer, API 5500, Sciex, Darmstadt, Germany or equivalent
- Chromatography column, Ascentis Express C18 2.7 µm, 50 mm x 2.1 mm id, or equivalent, Supelco, PA 16823-0048, USA, or equivalent
- Volumetric flasks, 10-mL, 20-mL, 100-mL
- Calibrated variable pipette, Eppendorf Multipette stream, Eppendorf AG, Hamburg, Germany or equivalent
- Brown glass bottles 20-mL, 50-mL
- Small instruments, e.g. Pasteur pipettes, autosampler vials, filter frits for reservoir

Reagents

- Methanol for chromatography, Optigrade, Promochem, Wesel, Germany, or equivalent
- Acetonitrile HPLC Gradient Grade, Code A/0627/17 Fisher Scientific, Schwerte, Germany, or equivalent
- Water, HPLC grade, purified with a Milli-Q-water system, Millipore Co., Eschborn, Germany, or equivalent
- Formic acid, Merck KGaA, Darmstadt, Germany, or equivalent
- Ammonium formate, Sigma Aldrich GmbH, Steinheim, Germany, or equivalent
- Nitrogen 5.0, 99.9990% purity, as curtain and collision gas, Linde AG, Höllriegelskreuth, Germany, or equivalent